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Full Length Research Paper

Growing the threatened seahorse *Hippocampus erectus* Perry 1810 in the laboratory

Rosana Beatriz Silveira[1] and José Rodrigo Santos Silva[1,2]

[1]Laboratório de Aquicultura Marinha-LABAQUAC/Projeto Hippocampus, Porto de Galinhas, Ipojuca-PE, Brazil.
[2]Departamento de Estatística e Ciências Atuariais, Universidade Federal de Sergipe, São Cristóvão-SE, Brazil.

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In Brazil, a seahorse pair of *Hippocampus erectus* was collected, and in captivity, mating and copulation occurred, followed by a pregnancy period of 12 days. The fry at birth had a mean height of 7.07 ± 0.947 mm and weight of 0.0037 ± 0.0049 g. The initial feed consisted of zooplankton up to the 10th day, and subsequently, they were fed nauplii and juveniles of *Artemia salina* and marine shrimp *Litopnaeus vannamei*. At 53 and 91 days of life, the animals had a mean height of 4.36 ± 0.315 and 6.03 ± 0.409 cm and mean weight of 0.377 ± 0.074 and 0.910 ± 0.172 g, respectively. At formation of the male's brood pouch, at 114 days of life, the mean height was 6.26 ± 0.374 cm and weight 0.9363 ± 0.1681 g. It was estimated that between 53 and 91 days of age, for every centimeter the seahorse grew, it gained about 0.3215 grams, where the equation height-weight obtained was $W = 0.00731\ L^{2.67728}$. Sexual maturity was reached at a height of 8.05 ± 0.56 cm. The size of the gestational period was 17.2 ± 6.70 days, and number of offspring produced was 377 ± 35.36 per pregnancy.

Keywords: diet, height-weight ratio, fertility, brood pouch, sex ratio, *Artemia salina*, *Litopenaeus vannamei*, Syngnathidae.

INTRODUCTION

In 1996, almost all species of seahorses (Syngnathidae: *Hippocampus*) were listed as "vulnerable" by the IUCN (International Union for Conservation of Nature), including the two Brazilian species recognized at the time: *Hippocampus reidi* and *H. erectus* (Figueiredo and Menezes, 1980; Rosa et al., 2002; Dias et al., 2002; Silveira, 2011). Currently, three Brazilian seahorse species are recognized: *H. reidi*, *H. erectus* and *H. patagonicus* (Silveira et al., 2014). This inclusion in the IUCN Red List, was due to excessive commercial fishing, degradation of their environment and the ignorance of their biology in natural or captive environments (Dias et al., 2002; Baum et al., 2003; Scales, 2010; Silveira et al., 2016). Seahorses are fish threatened with extinction worldwide and are among the most traded marine ornamental fishes, both aspects and for medicines and curios. However, like most marine ornamental fish, they are caught in the wild for trade because the demand is higher than the aquaculture industry can currently offer.

Corresponding author. E-mail: labaquac@yahoo.com

Corresponding author: R. B. Silveira, [1]Laboratório de Aquicultura Marinha-LABAQUAC/Projeto Hippocampus, Rua da Esperança, s.n., Porto de Galinhas, Ipojuca-PE, Brasil. E-mail: rosanasilveira@projetohippocampus.org

Global trade handles over 20 million seahorses per year, for the most varied purposes (Vincent, 1996), having reached in 2001 up to 67 tons per year, more than 23 million seahorses per year (Vincent et al., 2011). Although they are distributed all over tropical and the temperate regions worldwide (Lourieet al., 1999), 55 currently validated species (Froese and Pauly, 2016) are highly exploited, and only about 13 of the mare raised for trade (Koldewey and Martin-Smith, 2010). In 2004, CITES (International Convention on Trade in Endangered Species of Wild Fauna and Flora) included all species of seahorses in Appendix II of the Convention. The criteria of this Appendix (II) are not as restrictive as those of Appendix I, which prohibits fishing, but allows the sustainable use of resources. This decision put seahorses among the commercial marine teleosts most important to CITES (McPherson and Vincent, 2004). The purpose of CITES is to ensure that international trade does not exert unsustainable pressure on natural populations. Brazil is one of the 160 signatory countries of the Convention and is the major exporter of seahorses in Latin America, although the magnitude of this trade is not known in our country. According to 29 fishermen interviewed in Brazil, 25 said that the populations of seahorses declined around 86% (Vincentet al., 2011), but Brazilian data on these fishes are poorly known. For seahorses, there is no control or recording of commercial fishing in Brazil, with export data being insufficient to estimate the current catch of these fishes, and there is no specific protection law (Silveira, 2005). Rosa et al., (2006), working with the aquarist trade, determined the capture of 9,793 specimens of seahorses between 1997 and 2005, just in the state of Bahia, Brazil. In Pernambuco, Brazil, between 1999 and July 2000, 4,114 seahorses were exported by four companies registered with the Brazilian Institute of Environment and Renewable Natural Resources–IBAMA (Silveira, 2005). Recently, the three Brazilian species of seahorse were included in the Brazilian List of Endangered Species in the category "vulnerable" (MMA- Ordinance No. 445, 2014).

The trade of these fish is very lucrative for middlemen, who pay very little to the humble fishermen who live off or supplement their family income with this fishing. A live seahorse is sold in ornamental fish stores for about R$ 20.00, while the fisherman is paid R$ 1.00 for the "darker" and R$ 4.00 for the "colored", preferably red (Silveira, 2005). Fishing is done on any age or size, with no sparing of pregnant males. In open markets, for example in Recife, or Baia de Paranaguá, PR, one can find the trade of specimens of the three species occurring in Brazil, sold as dehydrated parts for use in "folk medicine", curios, lucky charms, etc., with price ranging between R$ 4.00 and R $15.00 per unit, depending on the size and skeletal condition (Silveira 2005, Silveira et al., 2014). In addition to the trade in ornamental fish and medicinal products, the animals suffer from environmental degradation and fishing by catch, especially with trawling (Salin et al., 2005; Foster and Vincent 2012; Vincent et al., 2011; Jardim et al., 2012; Filiz and Taskavak, 2012; Foster and Arreguin-Sánchez, 2013). To reduce the pressure on wild populations of seahorses, it has been proposed to breed them for trade (Jobet al., 2002; Koldewey and Martin-Smith, 2010).

Hippocampus erectus, despite its taxonomic uncertainty and distribution (Boehm et al., 2013; Silveira, 2011; Silveira et al., 2014) and all other threats common to the Syngnathidae and several other groups, such as trawling (Baumet al., 2003 ; Foster and Vincent, 2012), its culture has been demonstrated to be adequate and easy to manage to meet commercial and conservation needs (Linet al., 2009). In the coastal waters of Pernambuco, Brazil, the species is sighted with great difficulty, and over the past 13 years of research, our team found only six live specimens and three dead (one on the beach and two in trawls). Of the live specimens, only one was male. In this paper, we described the biology of *H.erectus* and its first rearing in captivity in Brazil.

METHODS

Collection, food and acclimatization of seahorses

Only one pair of seahorses of the species *H.erectus* was found and collected on the beach of Porto de Galinhas, Ipojuca, Pernambuco. The animals were not found together, they were caught one month apart. The seahorses underwent prophylactic treatment with 0.1% copper sulfate (Duijn, 1956) in individual quarantines and fed 10- to 14-day post-larval marine shrimp *Litopenaeus vannamei* (PLs 10-14) and adult *Artemia salina*. Afterwards, the seahorses were kept in a 60-laquarium containing natural sea water sterilized by chlorination, with salinity of 30, pH 8.3, photoperiod of 9h, biological filter plate and moderate aeration, in a room at 25 °C ± 1. The levels of ammonia, nitrite and nitrate were monitored weekly with colorimetric kits (Alcon, Brazil), and kept at 0mg/l. After six months of acclimatization in the laboratory, the male courted the female, resulting in mating between the pair.

Estimate of the gestation period and average brood produced

The development of six pregnancies of the same pair was monitored to determine the size of the gestational period and the average number fry expelled from the brood pouch at end of pregnancy.

Aquariums for newborns and juveniles

The birth of offspring, 100 fingerlings were transferred to four 100-l tanks (25 animals per tank, 0.25 ind/l), with external filter built with skeleton of calcareous algae *Halimeda* sp and parts of ceramic such as filter elements, and addition of skimmer and 11-W UV filter, with recirculating natural seawater. The water parameters were monitored every three days and maintained as with breeding tanks. In the water recirculation, 10% of the water was exchanged every other day until the animals were 3 months old, when they were transferred to two 500-l tanks fitted as the previous ones. At this stage, a *Halimeda* sp background was added and the tanks were planted with green seaweed. There are no more water changes, only fresh water replenishment for salinity maintenance.

Feeding newborns and juveniles

The initial feeding of newborns consisted of wild zooplankton collected in the sea and at low tide with150-mµ mesh net. The samples were collected, homogenized and diluted daily to120 ± 20ind/ml (mostly copepods and copepodites), providing 400ml of this dilution, twice daily (10:00and 16:00h). The photoperiod of the newborns was increased to12h, so that they could spend more time feeding. The seahorses consumed only wild zooplankton up to the 10^{th} day of life, and from the 11^{th} day and hence forth, nauplii of *A. salina* and *L. vannamei* (50% of each crustacean) were introduced at a concentration of 80± 20 ind/ml, with 500 ml of this mixture being offered twice daily (10:00 and16:00h). Feeding with zooplankton was continued for seven days to minimize the effect of diet change. After the second month of life, the seahorses were fed 5- to 7-day post-larval marine shrimp (PLs) and *A. salina* juveniles (0.5 cm long), and starting on the fourth month of life, 10-day PLs and *A. salina* adults.

Biometrics and weight

The offspring were weighed and measured at birth (n=40) and at 53 (n=50) and 91(n=84) days of life. The height (Ht), linear measurement from the top of the head to the tip of the stretched tail (Lourieet al., 1999) was made with a plastic ruler, which was submerged to avoid removing the animal out of the water. Weighing was done with a digital semi-analytical balance (0.001g), on which the animal was placed after the removal of excess water with tissue paper. Newborns, on the other hand, were weighed on an analytical balance (0.0001 g).

Height determination on brood pouch formation

The animals used for the calculation of mean height at brood pouch formation were juveniles from the smallest seahorse with fully formed pouch to the largest with the pouch still in formation. Juveniles with small pouch formation (values below the smallest male with fully formed pouch) were not considered for calculation because we wanted to determine the mean height of the seahorses with a fully formed pouch (Figure 1).

Statistical Analysis

The mean height at brood pouch formation was estimated by linear regression, using the various classes in height, and the variable indicating brood pouch formation assumed two values, "1" for pouch formed and "0" for pouch in formation.
The equation for the weight/height ratio was obtained by linear regression, which estimated the variation in weight according to height of seahorses, using a 5% significance level. The software used was R 3.2.2 (The R Core Team 2015).

RESULTS

Courting and copulation by *H. erectus* occurred during the day, where the male courted the female with characteristic moves, which were similar to those observed in this laboratory for *H. reidi*. Copulation occurred with the transfer of hydrated oocytes into the male's brood pouch, where they were fertilized. According to six consecutive pregnancies, the mean time for the species was 17.2 ± 6.70 days, and 377 ± 35.36 offspring resulted in each pregnancy. The fry were expelled through the orifice of the brood pouch after various and sequential contractions. The new borns had a mean height of 7.02 ± 0.43 mm and all exhibited a homogeneous light yellow color. They were fed from the first moment with wild zooplankton on without any difficulty and no deaths occurred. As of the11th day, nauplii of *L. vannamei* and *A. salina* were introduced as food, which were well accepted by the seahorses. From day 11 today 18, 16 animals died and were the only deaths recorded in three of the four experimental aquariums. On completing 53 days of life, the fish had a mean height of 4.36 ± 0.31 cm and wet weight of 0.377 ± 0.074 g (Fig. 2). The first brood pouch sign in males was recorded after 74 days when there was a different color in the first ring of the ventral surface of the tail. After 91 days, the animals exhibited a height of 6.03 ± 0.409 cm and weighed 0.910 ± 0.172 g, but no pouch was fully formed, which occurred at 107 days. The formation of the male brood pouch occurred at a mean height of 6.26 cm, and weight of 0.9363 ± 0.1681 g. Data analysis demonstrated that 95% of males of this species begin brood pouch formation between 5.90 and 6.10 cm, while the fully formed pouch appears between 6.024 and 6.498 (Table 1). The sex ratio of off spring was 1:1, where 40 males and 44 females were observed ($Z = 0.4364$, $p = 0.6631$).

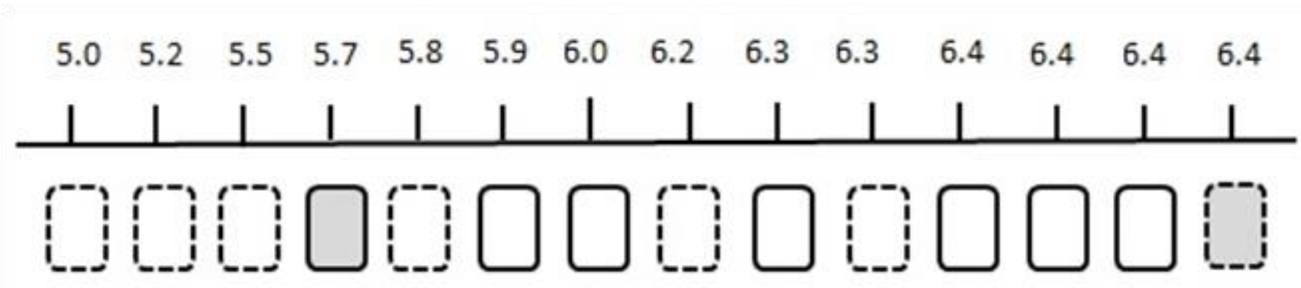

Figure 1. Illustrative scheme of the criteria used in selecting the data to estimate the average height brood pouch formation of males: brood pouch in formation; smaller male with brood pouch fully formed; brood pouch fully formed; larger male with brood pouch in formation; the numbers represent the heights (cm).

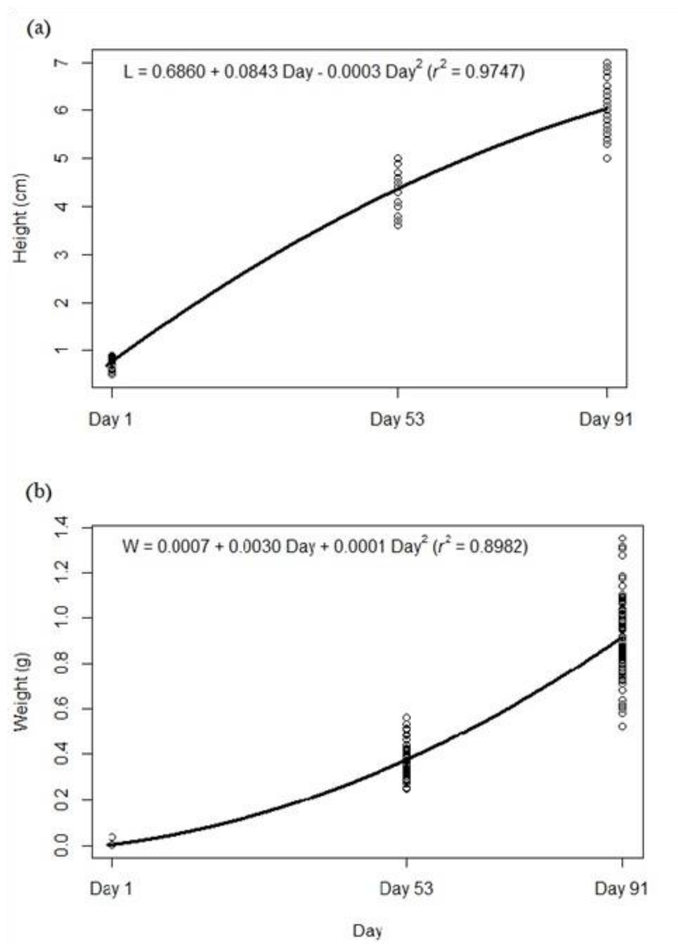

Figure 2. Height (a) and wet weight (b) of the seahorse *Hippocampus erectus* cultivated from birth to 91 days of life (n = 84).

Considering a linear model for the weight x height equation, $W = -1.024 + 0.3215 \times H_t$, it was estimated that during the intense growth period (between 53 and 91 days of age), for every centimeter the seahorse grew, it gained about 0.321 g. The general equation obtained for the height-weight ratio, $W = 0.0067H^{2.7247}$ (adjusted $r^2 = 0.9863$, n = 174, $p < 2.2e^{-16}$), showed that in this phase, all subjects had negative allometric growth (Fig. 3). All juveniles during the second month of life developed dermal appendages, which persisted in adulthood but

Table 1: Height at formation of the brood pouch in the *Hippocampus erectus* male.

	Coefficients	Standard Error	t	P	Lower 95%	Upper 95%
Interseption*	6.017	0.056	108.419	0.000	5.906	6.129
Pouch**	0.244	0.118	2.065	0.043	0.007	0.481

*Mean height of male brood pouch in formation (cm); ** Mean difference between the animals with pouch formed versus pouch in formation (cm). The average height of animals with fully formed pouch is given by the sum of the coefficients 6.017 + 0.244 = 6.261

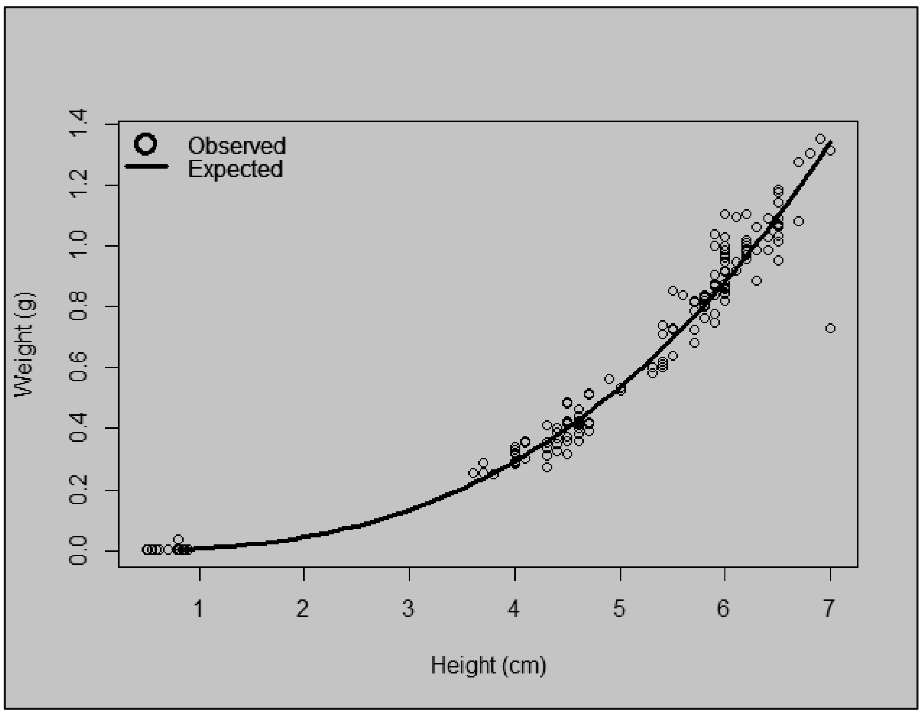

Figure 3. Height-weight ratio obtained for cultured *Hippocampus erectus* until day 91(n = 84).

decreased with age (Fig. 4). When sexual maturity was reached at 8.05 ± 0.56 cm, courtship and copulation began between pairs, but there was a high degree of promiscuity with males courting males, even in the presence of females.

DISCUSSION

According to Foster and Vincent (2004), the majority of seahorse species exhibit monogamous behavior, but there is an apparently low level of polygamy recorded for *H. abdominalis*, *H. subelongatus*, *H. fuscus* and *H. guttulatus*, and there was one polygamous event for *H. reidi* (Silveira, 2009). Courting and mating between males and females of *H. erectus* in our study followed the pattern described for the genus (Strawn, 1958; Mason jones and Lewis, 1996; Garrick-Maidonent, 1997; Foster and Vincent, 2004; Lin et al., 2008; Silveira, 2009; Rosenqvist and Berglund, 2011). However, after the offspring reached adulthood and started reproductive activities (at a height of about 8.0 cm), there was a high level of promiscuity with males courting males, even in the presence of the same number of females

Figure 4. Young of *Hippocampus erectus* cultivated for 150 days.

(1:1).These observations were very intriguing, as it involved choices made by the males repeatedly for many days. A similar situation had only been observed for *H. reidi,* but that was induced by the unique isolation of males (Silveira, 2009).
The mean height (Ht) of cultured *H. erectus* at birth has been reported to be 15.9 mm (Herald and Racowiks, 1951), 16.4 ± 1.8 mm (Vite-Garcia et al., 2014), 13.0 mm standard length (SL) (Chung et al.,1989) and 11.3 ± 0.06 mm (SL) (Lin et al., 2008). In the present study, *H. erectus* had a mean height of 7.02 ± 0.43 mm at birth, smaller than that reported by the above cited authors.
According to Vincent and Giles (2003), the juvenile size in the same species can vary with latitude and environmental variables, with photoperiod and temperature also being factors, where higher latitudes result in increased size of egg and juvenile at birth. In fact, the difference in size at birth between *H. erectus* grown in Pernambuco (8ºS) and other countries such as the US (23ºN, Florida; 37ºN, San Francisco) could be explained by this condition, and this probably would be due to the need for greater gain in energy reserves and fat for protection against the low temperatures that occur at high latitudes. Though growing up in captivity under controlled conditions, characteristics are preserved due to genetic baggage responsible for the adaptation of each community to its environment over millions of years (Freeman and Herron, 2009).
In our work, *H. erectus* measured 4.36 ± 0.31 cm (Ht) at 53 days of life, with an mean weight of 0.377 ± 0.074 g, values similar to those of 4.34 ± 0.2 cm and 0.249 ± 0.053 g found by Vite-Garcia et al., (2014) at 60 days of life for the same species, fed on a diet *Artemia* sp. enriched with Selco®, where in this phase, 7 days or more of culture can produce a large difference in final mean height. Herald and Racowiks (1951) found 5.08 cm for *H. erectus* at 60 days of life. According to Lin et al., (2008), *H. erectus* had a standard length (SL) of 6.32 ± 0.52 cm at 67 days and wet weight was 0.45 ± 0.12 g.
In the third month, the heights were very similar, 6.35 cm and 6.03 ± 0.401 cm for Herald and Racowiks (1951) and this work, respectively. Regarding the formation of the male brood pouch, Chung et al., (1989) observed at10.0 cm (SL), corresponding to an age of three months, the initial formation of this structure. Herald and Racowiks (1951) reported the formation of the brood pouch between 6.35 to 7.0 cm in height, corresponding to 3.5 months, while Scarratt (1995) estimated the formation of this structure between 5 and 6 months for *H. erectus*. For *H. guttulatus*, Cartagena (2014) observed the first sign of pouch formation at 124 days of life (4 months) with 8.7 cm in height. In this work, the first sign of a brood pouch (different color on the ventral side of the first caudal rings) appeared at 74 days of life (2.46 months), where the mean

height at brood pouch formation was 6.26 cm, corresponding to 3.5 months of age. In situ data showed *H. erectus* with a formed brood pouch at 4.0 cm in height (Teixeira and Musick, 2001).

In this work, after following six consecutive pregnancies of the same parents, the mean length of gestation was determined to be 17.20 ± 6.70 days, but by observing the exact day of three clutches inside the male brood pouch (of the six recorded) until the day of birth, two showed 12 days and one 13. For the other three clutches, the exact date of the transfer of eggs was not observed. Thus, it is possible that the average gestational period was overestimated and that *H. erectus* had the same gestation period as *H. reidi* in Pernambuco (12 days) (Silveira and Fontoura, 2010). The mean number of offspring produced in the six pregnancies was 377 ± 35.36 juveniles. Lin et al., (2008) estimated for *H. erectus* (six pairs) a gestational period of 17.33 ± 2.94 and offspring of 272.33 ± 66.45 juveniles per brood pouch. Lin et al., (2008) reported for their cultivation conditions a survival rate of 71.11 ± 10.18% up to nine weeks of culture at 28 °C, Vite-Garcia et al., (2014) obtained 82% survival in the culture of *H. erectus* up to 60 days of live, at 26 ± 1 °C, while in this work, we observed 84% survival at 150 days of life at 25 ± 1 °C. Hora and Joyeux (2009) used a diet consisting of wild zooplankton, *A. salina* enriched with Super Selco® and the mysid *Misidyum gracile* (live and frozen), and obtained 88.3% survival in *H. reidi* culture. Pham and Lin (2013) achieved a survival rate of 84.3% for *H. reidi* up to 28 days of live using a diet of *Artemia* enriched with Dan's Feed® and 74.5% with a diet of wild plankton, in both cases stocked at 1 ind/l. Zang et al., (2010) stocked *H. erectus* at 1 ind/L and obtained 87.8% survival up to 40 days of life.

The relationship between estimated weight and height in this work, $W = 0.0067\ H^{2.7247}$ (adjusted $r^2 = 0.9863$, $n = 174$, $p < 2.2e^{-16}$) was similar to that obtained by Linet al., (2008), $W = 0.0034L^{2.5535}$ ($r^2 = 0.9903$, $n = 12$, $p < 0.01$), although we observed a faster weight gain in our cultures, possibly due to the quality of food provided (wild zooplankton, *L. vannamei* nauplii and post-larvae fed artificial diet, *A. salina* nauplii, juveniles and adults enriched with microalgae in this work versus *A. salina* nauplii, juveniles, adults enriched with microalgae and fish oil emulsion-Super Selco®, and frozen *Mysis* spp. in Lin et al., 2008). Palma et al., (2012) observed better development for *H. guttulatus* fed with the shrimp *Palaemonetes varians*, with artificial diet for 12 weeks, recording the number of pregnancies, greater number of fingerlings incubated and greater size on birth also.

According to Teixeira and Musick (2001), the fertility of *H. erectus* in Chesapeake Bay, FL was 451 ± 232.10 embryos per pregnancy, with a maximum of 1552, suggesting that there is the possibility to optimize the reproductive capacity of the species in culture, by working with selected specimens, proper nutrition and enriched diet.

We believe that the aquaculture of endangered species, especially those with high commercial appeal, such as seahorses and many other ornamental fishes and marine invertebrates, should be encouraged as a conservation measure for the species in their environment.

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